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PREDNISOLONE BLOCKS EXTREME INTERMALE SOCIAL AGGRESSION IN SEIZURE-INDUCED, BRAIN-DAMAGED RATS: IMPLICATIONS FOR THE AMYGDALOID CENTRAL NUCLEUS, CORTICOTROPHIN-RELEASING FACTOR, AND ELECTRICAL SEIZURES [1]

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Summary.—In two separate blocks of experiments, the extreme within-group aggression which is typically associated with limbic seizure-induced brain injury in male rats was attenuated or abolished within two days by the administration of prednisolone in the water supply. The effect was specific to the aggression and was not simulated by dexamethasone. The results support the hypothesis that interference with inhibitory inputs to the central nucleus of the amygdala and the enhanced stimulation by corticotrophin-releasing factor facilitates physical aggression within groups of male rats. Potential relevance to curbing aggression ("conflict") between groups of male humans is discussed.

Aggression within a species is considered a primary component of selection and adaptation (Lorenz, 1966; Wilson, 1975). The central nucleus of the amygdala has been implicated as a significant modulator of intermale aggression for rodents (Kaada, 1972). We have reported that rats in which chronic limbic seizures have been induced by a single injection of lithium and pilocarpine have *always* exhibited discernable damage in all subgroups of amygdaloid nuclei except within the central nucleus (Persinger, Bureau, Kostakos, Peredery, & Falter, 1993). Our major hypothesis is that damage to the inhibitory (basolateral group) modulators of the central group would disinhibit its function. These male rats should and do develop extreme within-group aggression which requires social separation to prevent fatalities.

The central nucleus of the amygdala contains copious concentrations of receptors for corticotrophin-releasing factor (Koob, Heinrichs, Menzaghi, Pich, & Britton, 1994). Either electrical lesions of this nuclear group or administration of a glucocorticoid antagonist, prednisolone, attenuates the acoustic startle response whose integrity is primarily dependent upon a pathway between the suprageniculate nucleus of the thalamus and the central amygdala (Liang, Melia, Campeau, Falls, Miserendino, & Davis, 1992). We predicted that, if the extreme group aggression were due to the disinhibited electrical activity of the central nucleus because of the epileptogenic nature

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of corticotrophin-releasing factor (Baram & Schultz, 1991; Ehlers, Henriksen, Wang, Rivier, Vale, & Bloom, 1983), then administration of a possible inhibitor of corticotrophin-releasing factor (Tazi, Dantzer, Le Moal, Rivier, Vale, & Koob, 1987) such as prednisolone (which binds to type 1 and type 2 glucocorticoid receptors) should attenuate or block the aggression specifically without potentially correlative confounding effects (such as a generalized decrease in activity). Compounds, such as dexamethasone, which are sequestered primarily by type 2 glucocorticoid receptors (Eckland, Harbuz, Jessop, & Lightman, 1991), should not affect the aggression and hence could be employed as a control for nonspecificity.

Method

To test this hypothesis two experiments were designed and then compared after approval by the university ethics committee. The first block experiment involved 12 180-day-old male albino Wistar rats (Charles River Breeding, Quebec). About three months before the experiment began, nine of the rats had been injected with 3 mEq/kg of lithium chloride and then 4 hr. later with 30 mg/kg of pilocarpine. To facilitate survival each rat had been injected with 30 mg/kg of acepromazine (Atravet) 30 min. after the onset of the limbic forelimb clonus (all injections were subcutaneous). The other three rats were the same age and served as controls. Both rats in which the seizures had been induced and the control rats were housed singly for 10 days before and throughout the duration of the experiment.

Each day, for 48 successive days, the same triad of rats were removed from their home cages and placed in plastic (51-cm by 41-cm by 20-cm) cages for 1 hr. There were a total of three triads of rats who had sustained the seizure-induced amygdaloid injury and one triad of rats which served as controls. The following observations were recorded per cage (the sum for all three rats) per hour: number of different episodes of boxing (an upright stance with forepaw contact but not biting), total duration of boxing, numbers of squeaks, and numbers of bites per hour. During Days 11 through 14 and 30 through 35, all rats received 0.5 mg/cc of prednisolone orally (*ad libitum*) through their water supply in the home cage. During Days 21 through 24 and 40 through 44, 1 mg/cc of dexamethasone was delivered through the water supply. The remaining days served as tap-water reference (control) periods.

In Exp. II, a single group of four albino male Wistar rats had been housed together in a 140-cm by 37-cm by 37-cm plastic cage for approximately 150 days after they had received the treatment for induction of limbic seizures when they were 80 days of age. The room temperature was regulated (20 to 22° C) and the light:dark cycle was 0730 to 1930 hr. with light onset at 0730 hr. Activity was monitored by the numbers of interruptions of

one of three photocells (Radio Shack Model 49-551) which were spaced equally along the long axis of the cage. Squeaks were recorded by a Lafayette Model 63040 2-channel Voice Reaction Time Sensor. The numbers of squeaks per hour has correlated significantly ($r > .90$) with the presence of blood on the cage walls and ($r = .81$) with the visual observations of number of body contacts per hour (2300 hr. to 2400 hr.) during 10 successive nights. The total numbers of squeaks and activity were added every 10 min. and stored on a personal computer. To minimize contributions from cultural noise, the mean numbers of photocell disruptions (activity) and indicators of aggression (squeaks) were obtained for 0030 hr. and 0630 hr. (local nighttime).

After about two to three months of intermittent frequent episodes of hypersexuality similar to those reportedly previously (Persinger, 1994), extreme intragroup aggression began to emerge as inferred by blood stains along the cage wall and multiple wounds on the rats' snouts and ears. Close scrutiny during nighttime (red light) observations indicated that many of the most vicious attacks began when the rats which were attacked had displayed odd, rapid movements of the pinnae (which we have presumed to be associated with paroxysmal electrical activity within the suprageniculate nucleus). Given the extreme aggression and laceration and to remain in compliance with the endpoints of the protocol as defined by the guidelines of the Canadian Council on Animal Care, the rats were separated for two days. Following regrouping, as the escalation of aggression occurred again, the optimal treatment as derived from Exp. I (prednisolone, 1 mg/cc within the water supply) was administered for five days.

For Exp. I multiple regression analyses were completed. Dummy variables for each group of rats were coded such that the days of treatment were attributed a value = 1 while the remaining days were equal to 0 (thus three independent variables, i.e., water, prednisolone, and dexamethasone). Days were employed as cases. Accommodation for serial correlations (drifts in slopes of the behaviors over time) were completed. The dependent variables were the numbers of bites, boxing episodes or squeaks per session (day), and the total duration of boxing stances per session. For Exp. II, one-way analyses of variance were completed by partitioning the baseline, treatment, and posttreatment periods into a group variable. Although this procedure is not commensurate with the assumption of independence between cases (days), we assumed that this violation would have minimal consequences upon a large effect size. All analyses involved SPSS software on a VAX 4000 computer.

Results

The results of Exp. I are shown in Fig. 1 (upper panel) which depicts

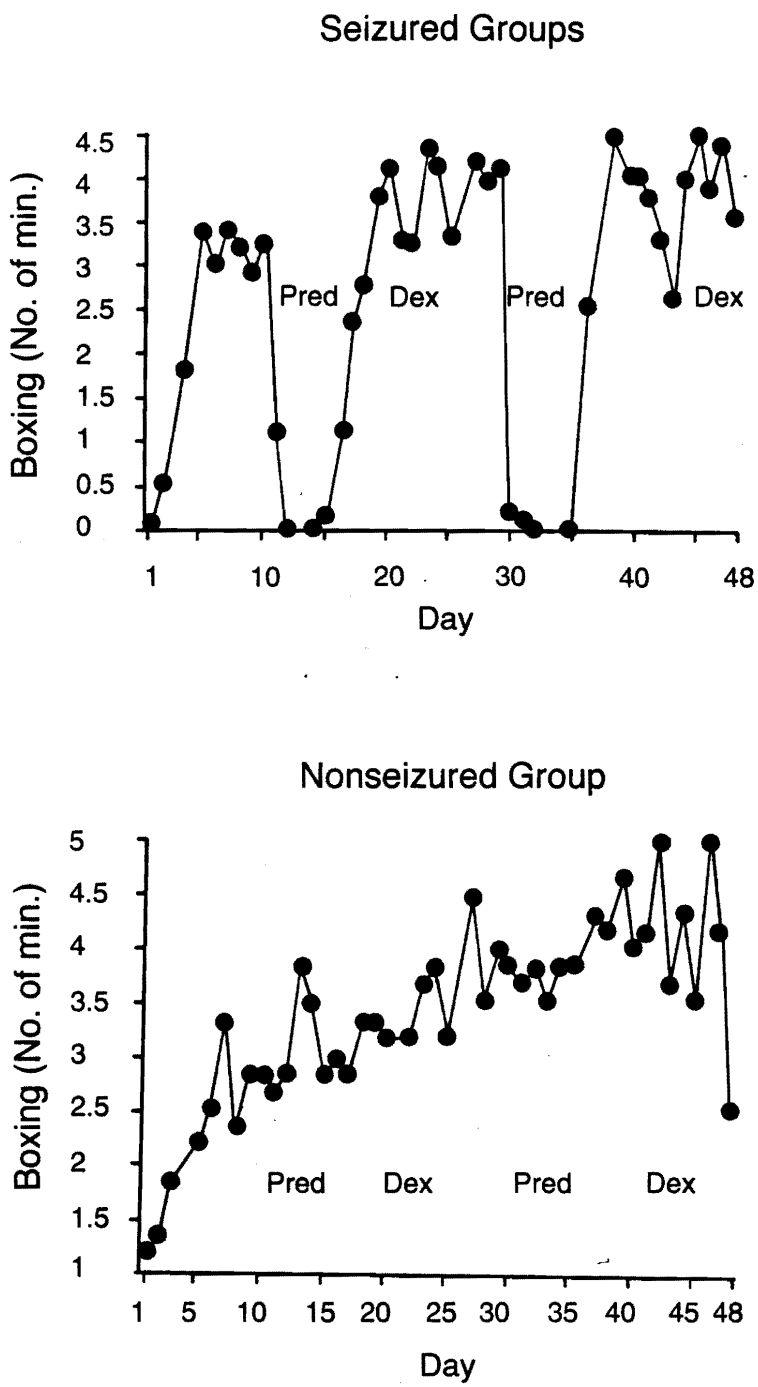

FIG. 1. Mean numbers of minutes of boxing (aggressive) behavior per hour per day for groups of rats as a function of oral (water supply) treatment with prednisolone (0.5 mg/cc) or dexamethasone (1 mg/cc). Upper panel: rats with histories of chronic limbic epilepsy; bottom panel: controls.

the mean daily variation in the total duration of boxing (total minutes per hour) for all three triads of rats (combined) in which seizures had been induced. Oral prednisolone was associated with an attenuation of boxing (the results were similar for bites but less evident for squeaks). This inference of

aggression was not attenuated when the rats received water or dexamethasone in the water supply. For each of the three groups of rats the intergroup consistency, as inferred by the multiple *r* values, ranged between .59 and .64. As can be seen in Fig. 1 (lower panel), neither dexamethasone nor prednisolone treatment altered the boxing behavior of the control rats even though the absolute incidence of this behavior was similar to that of the experimental animals. Although the control rats exhibited squeaks and some body contact, the absolute incidence was at least one order of magnitude less than that of the rats in which seizures had been induced. In addition, control rats never inflicted tissue damage upon other members of the group.

The results of Exp. II are shown in Fig. 2. Visual inspection indicated that the physical aggression as inferred from the numbers of squeaks per unit time within the group decreased following the administration of oral prednisolone (1 mg/cc) through the water supply while general activity was not conspicuously altered. Given the heterogeneity of variance, square-root transformations were completed for the daily indices of the numbers of squeaks. One-way analyses of variance, in which were compared the baseline (excluding the two days when the rats were removed), treatment days, and

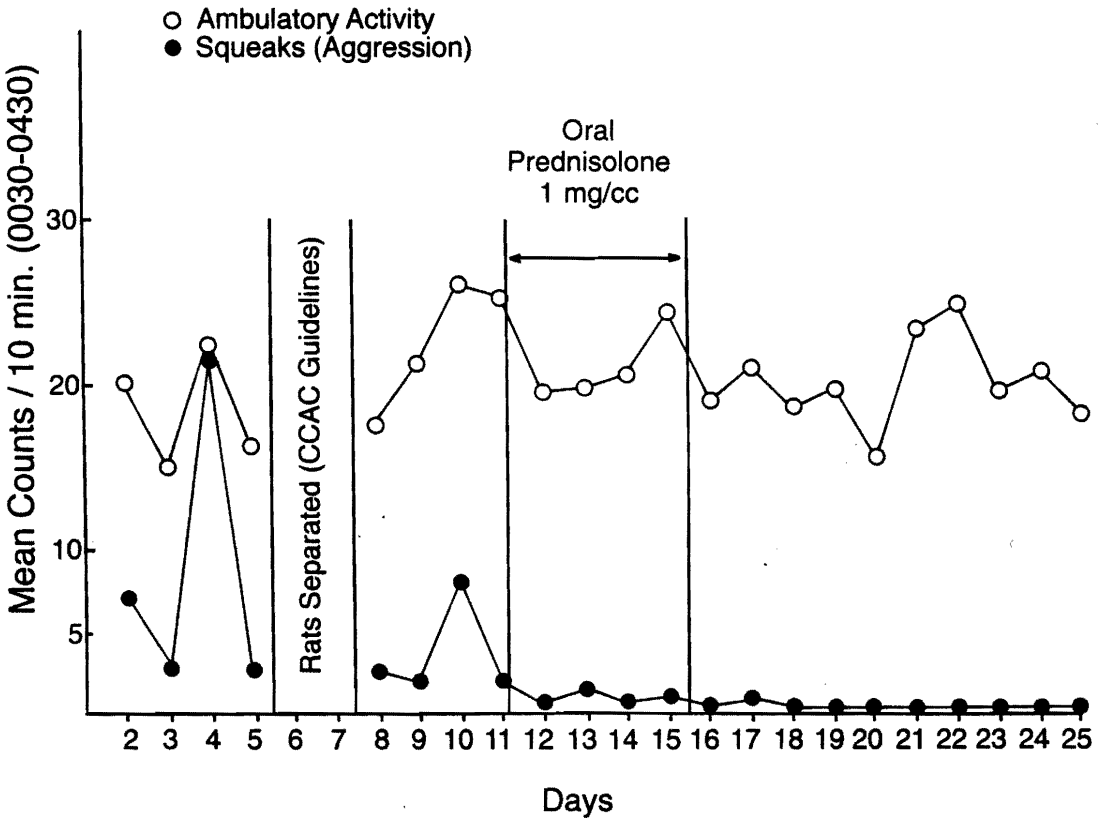

FIG. 2. Mean numbers of squeaks (closed circles) and of general ambulatory activity (open circles) per unit time (between 0030 and 0430 hr. local time) before, during, and after continuous treatment in the water supply with 1 mg/cc of prednisolone

posttreatment days verified the observations that there was a significant decrease in the amount of aggressive behavior ($F_{2,25}=8.81$, $p<.001$; $eta=0.64$). *Post hoc* (Tukey) analysis indicated that the effect was due primarily to the decrease in numbers of squeaks between the baseline and the treatment and posttreatment periods which did not differ significantly from each other. Nonparametric analysis of variance (Kruskal-Wallis) confirmed the statistically significant ($\chi^2=10.74$, $p<.01$) decrease in the numbers of squeaks. There were no significant ($F_{2,25}=1.45$, $p>.05$) differences among the three periods with respect to general ambulatory activity. Covariance for daily ambulatory activity before the analyses increased the strength of the effect ($F_{2,24}=12.20$, $p<.001$; $eta=0.75$) of the treatment upon the daily incidence of aggression.

Discussion

The results of this study were qualitatively clear and supported the present and previous hypothesis that stimuli that should enhance the effects of corticotrophin-releasing factor (or some correlative neuropeptide) within the central nucleus of the amygdala also accentuate aggression toward another male (Herbert, 1993). The protracted intermale aggression of rats within which limbic damage has been induced by protracted seizure activity may involve processes correlated with both the transmitters involved with the receptors which sequester corticotrophin-releasing factor and with the enhanced (disinhibitory) activity within the central nucleus of the amygdala. The specificity of the effect was shown by the absence of influence by dexamethasone on both inferences of aggression and ambulation during Exp. I and by the absence of attenuation in general activity but not in aggression during Exp. II. The counterargument that the decreased aggression was due to a generalized tranquilizing or "stress" effect from the drug treatment is unlikely.

The major discrepancy of this study, the observation of reversibility of the prednisolone treatment for the hourly daily group exposures (Exp. I) relative to the permanent attenuation for the rats housed together chronically, is not clear. The major caveat to the conclusion that the amygdala was the primary site assumes that the other correlative areas of neuronal necrosis did not generate a unique neural matrix. In addition, the work by Makino, Gold, and Schulkin (1994) suggested that subtle differences in the dosages of corticosteroids can produce both transient and persistent (several weeks) alterations in the amount of corticotrophin-releasing factor within the central amygdaloid nucleus.

Lorenz's concept of aggression emphasizes the utility and importance of aggression for the maintenance of group integrity and for ensuring social interaction. However, the extreme development of adaptive strategies, of

which aggression could be considered a primary form, could also contribute to the extinction of a species. Despite the "logical" arguments concerning the marked cerebral difference between male homosapiens and male rodents, the intricate receptor systems and microstructural organization are remarkably similar (Mendelsohn & Paxinos, 1991).

Although there may be disconcordance for the specific form of the aggressive display (biting vs bombing members of the same species), the general behavioral patterns which are associated with disinhibition of this structure may be similar. Any stimuli, from dietary input to social ritual, which facilitate the occurrence of central amygdaloid activation in context of the chemistry of psychosocial stress, would increase the probability of physical aggression. The results of this study suggest that introduction of specific inhibitory compounds within the water supply could attenuate this aggression.

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